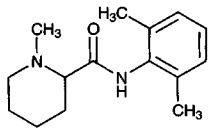
Mepivacaine



Molecular formula: $C_{15}H_{22}N_2O$
Molecular weight: 246.35
CAS Registry No.: 96-88-8, 1722-62-9 (HCl)
Merck Index: 5905
Lednicer No.: 1 17

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 25 µL EtOH + 25 µL 17.6 µg/mL ropivacaine in EtOH, mix. Add 5 mL diethyl ether, vortex for 3 min, centrifuge at 2000 g for 10 min at 4°. Add 500 µL 100 mM hydrochloric acid to organic phase, back extract for 3 min. Discard organic phase, add 5 mL n-pentane containing 100 µL isoamyl alcohol, wash for 3 min, discard organic phase again. Add 50 µL 2 M NaOH, extract aqueous phase with 5 mL n-pentane containing 100 µL isoamyl alcohol, centrifuge at 2000 g for 10 min at 4°. Evaporate organic phase to dryness under a gentle stream of nitrogen at 40°. Reconstitute the residue in 100 µL mobile phase, inject a 30 µL aliquot.

HPLC VARIABLES

Guard column: 10 × 4.0 40 µm α1-AGP (J.T.Baker)
Column: 100 × 4.0 5 µm α1-AGP (J.T.Baker)
Mobile phase: 2-Propanol:buffer 6.8:93.2 (Buffer was 3.6 g/L $Na_2HPO_4.12$ H2O adjusted to pH 6.8 with phosphoric acid.)
Column temperature: 30
Flow rate: 1.1
Injection volume: 30
Detector: UV 210

CHROMATOGRAM

Retention time: 4.6 (R-(-)), 5.8 (S-(+))
Internal standard: S-(-)-ropivacaine (10.5)
Limit of detection: 3 ng/mL
Limit of quantitation: 5 ng/mL

OTHER SUBSTANCES

Noninterfering: bupivacaine
Interfering: lidocaine, prilocaine

KEY WORDS

plasma; chiral; pharmacokinetics

REFERENCE

Vletter,A.A.; Olieman,W.; Burm,A.G.L.; Groen,K.; van Kleef,J.W. High-performance liquid chromatographic assay of mepivacaine enantiomers in human plasma in the nanogram per milliliter range, *J.Chromatogr.B*, **1996**, *678*, 369–372.

SAMPLE

Matrix: blood
Sample preparation: Condition a 1 mL Bond-Elut C18 SPE cartridge with 1 mL 100 mM HCl, 1 mL MeOH and 1 mL water. Add 350 µL 100 mM pH 4.5 phosphate buffer and 40 µL 200 µg/mL S-bupivacaine in MeOH to 1 mL serum, vortex. Add the mixture to the SPE cartridge and pass it through the cartridge attached to a vacuum manifold, wash 4 times with 250 µL water, then wash with 500 µL MeCN and allow to air dry for 1 min between each wash. Elute with four 250 µL aliquots of MeOH containing 2% HCl, evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 1 mL mobile phase and inject a 100 µL aliquot.

HPLC VARIABLES

Column: 250 × 4 5 µm Sumichiral OA-4700 (YMC, Wilmington, NC, USA)

Mobile phase: Hexane:dichloroethane:absolute MeOH 85:10:5
Flow rate: 0.8
Injection volume: 100
Detector: UV 220

CHROMATOGRAM
Retention time: 9.3 (R-(+)), 11.4 (S-(-))
Internal standard: S-bupivacaine (7.7)
Limit of detection: 100 ng/mL
Limit of quantitation: 150 ng/mL

KEY WORDS
chiral; SPE; serum

REFERENCE
Siluveru,M.; Stewart,J.T. Stereoselective determination of mepivacaine in human serum using a bush-type chiral stationary phase and solid-phase extraction, *J.Chromatogr.B*, **1997**, *690*, 359–362.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 50 μL 40 μg/mL etidocaine hydrochloride in water + 100 μL 1 M NaOH, vortex for 15 s, add 5 mL diethyl ether, shake on a reciprocating shaker for 10 min, centrifuge. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μL mobile phase, inject a 80 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.2 10 μm μBondapak C18
Mobile phase: MeCN:50 mM pH 5.80 Na_2HPO_4 25:75
Flow rate: 0.9
Injection volume: 80
Detector: UV 210

CHROMATOGRAM
Retention time: 3.7
Internal standard: etidocaine (12.0)

OTHER SUBSTANCES
Extracted: 2,6-pipecolylxylidine, bupivacaine, lidocaine
Noninterfering: metabolites, 2,3-chloroprocaine, theophylline, mexiletine, quinidine, disopyramide, verapamil, phenobarbital, phenytoin, carbamazepine, ethosuximide, digoxin, theobromine, caffeine, furosemide, phenprocoumon, aldactone

KEY WORDS
plasma

REFERENCE
Ha,H.-R.; Funk,B.; Gerber,H.R.; Follath,F. Determination of bupivacaine in plasma by high-performance liquid chromatography, *Anesth.Analg.*, **1984**, *63*, 448–450.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL 1 M NaOH + 3 mL heptane:ethyl acetate 90:10, shake for 2 min, centrifuge at 1200 g for 10 min. Remove the organic phase and add it to 50 μL 50 mM sulfuric acid, shake for 2 min, centrifuge at 1200 g for 5 min. Remove the aqueous phase and add it to 820 μg sodium acetate, inject a 40 μL aliquot. (The sodium acetate was measured out by adding 50 μL 200 mM sodium acetate in MeOH to the tube and evaporating the MeOH.)

HPLC VARIABLES
Column: 250 × 4 10 μm μBondapak C18
Mobile phase: MeCN:10 mM NaH_2PO_4 7:93, adjusted to pH 2.1

Column temperature: 30
Flow rate: 1
Injection volume: 40
Detector: UV 205

CHROMATOGRAM
Retention time: 15
Limit of detection: 5 ng/mL

OTHER SUBSTANCES
Extracted: articaine

KEY WORDS
plasma; rabbit

REFERENCE
Le Guévello,P.; Le Corre,P.; Chevanne,P.; Le Verge,R. High-performance liquid chromatographic determination of bupivacaine in plasma samples for biopharmaceutical studies and application to seven other local anaesthetics, *J.Chromatogr.*, **1993**, *622*, 284–290.

SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL BondElut C18 SPE cartridge once with 1 M HCl, twice with MeOH, and once with water, remove the liquid completely with suction each time. Add 250 μL bupivacaine in 100 mM NaH_2PO_4 and 250 μL serum to the column at 1 mL/min, wash twice with water and once with MeCN draining the column completely after each wash, elute with 250 μL eluting solution, centrifuge for 20 s to remove last of eluate, inject a 5 μL aliquot of the eluate. (Eluting solution was 2.5 mL 35% perchloric acid in 100 mL MeOH.)

HPLC VARIABLES
Guard column: 15 × 3.2 7 μm RP-8 (Applied Biosystems)
Column: 150 × 4.6 5 μm Ultrasphere octyl
Mobile phase: MeCN:10 mM KH_2PO_4 25:80, pH 5.2
Flow rate: 1.5
Injection volume: 5
Detector: UV 205

CHROMATOGRAM
Retention time: 2.6
Internal standard: bupivacaine (7.2)

OTHER SUBSTANCES
Extracted: bupivacaine, meperidine, fentanyl
Noninterfering: acetaminophen, codeine, epinephrine, morphine, diazepam

KEY WORDS
serum; SPE

REFERENCE
Gupta,R.N.; Dauphin,A. Column liquid chromatographic determination of bupivacaine in human serum using solid-phase extraction, *J.Chromatogr.B*, **1994**, *658*, 113–119.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 264

CHROMATOGRAM
Retention time: 4.54
Limit of detection: <120 ng/mL

KEY WORDS
whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE
Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7

Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 1.7

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE
Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions
Sample preparation: Inject an aliquot of a 200 μM solution in MeOH.

HPLC VARIABLES

Column: 100 × 4.7 7 μm Hypercarb (Shandon)
Mobile phase: MeOH containing 5 mM N-benzyloxycarbonylglycyl-L-proline and 4.5 mM NaOH
Column temperature: 17
Injection volume: 20
Detector: UV 270

CHROMATOGRAM

Retention time: k' 2.32 (first enantiomer)

KEY WORDS
chiral; α = 1.11

REFERENCE
Huynh,N.-H.; Karlsson,A.; Pettersson,C. Enantiomeric separation of basic drugs using N-benzyloxycarbonyl-glyclyl-L-proline as counter ion in methanol, *J.Chromatogr.A*, **1995**, *705*, 275–287.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

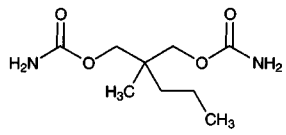
CHROMATOGRAM
Retention time: 7.71 (A), 4.20 (B)

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

Meprobamate



Molecular formula: $C_9H_{18}N_2O_4$
Molecular weight: 218.25
CAS Registry No.: 57-53-4
Merck Index: 5908
Lednicer No.: 1 218

SAMPLE
Matrix: blood
Sample preparation: 500 µL Serum + 100 µL 200 mM HCl + 200 µL 100 µg/mL carisoprodol + 1 mL chloroform, shake for 2 min, sonicate for 1 min, centrifuge for 1 min. Remove the organic layer and add it to 200 mM NaOH, shake for 1 min, sonicate for 1 min, centrifuge for 1 min. Remove 750 µL of the organic layer and evaporate it to dryness, dissolve the residue in 50 µL mobile phase, inject a 20 µL aliquot.

HPLC VARIABLES
Column: 100 × 3 octyl CP-tm-Spher C8 glass column (Chrompack)
Mobile phase: MeCN:50 mM NaH_2PO_4 35:65 adjusted to pH 2.2 with phosphoric acid
Flow rate: 0.8
Injection volume: 20
Detector: UV 190

CHROMATOGRAM
Retention time: 1.8
Internal standard: carisoprodol (5.5)
Limit of detection: 2000 ng/mL

KEY WORDS
serum

REFERENCE
Van Damme,M.; Molle,L.; Abi Khalil,F. Useful sample handlings for reversed phase high performance liquid chromatography in emergency toxicology, *J.Toxicol.Clin.Toxicol.*, **1985**, *23*, 589–614.

SAMPLE
Matrix: blood
Sample preparation: 500 µL Serum + 100 µL 200 mM HCl + 200 µL 100 µg/mL carisoprodol + 1 mL chloroform, shake for 2 min, sonicate for 1 min, centrifuge for 1 min. Remove the organic layer and add it to 200 mM NaOH, shake for 1 min, sonicate for 1 min, centrifuge for 1 min. Remove 750 µL of the organic layer and evaporate it to dryness, dissolve the residue in 50 µL mobile phase, inject a 20 µL aliquot.

HPLC VARIABLES
Column: 100 × 3 octyl CP-tm-Spher C8 glass column (Chrompack)
Mobile phase: MeCN:50 mM NaH_2PO_4 35:65 adjusted to pH 2.2 with phosphoric acid
Flow rate: 0.8
Injection volume: 20
Detector: UV 190

CHROMATOGRAM
Retention time: 1.8
Internal standard: carisoprodol (5.5)
Limit of detection: 2000 ng/mL

KEY WORDS
serum

REFERENCE
Hormazabal,V.; Steffanak,I.; Yndestad,M. Simultaneous extraction and determination of sulfadiazine and trimethoprim in medicated fish feed by high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *648*, 183–186.

SAMPLE
Matrix: formulations
Sample preparation: Powder tablets, weigh out amount equivalent to 100 mg meprobamate, dissolve in 50 mL mobile phase, sonicate for 10 min, filter, inject an aliquot.

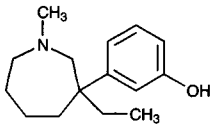
HPLC VARIABLES
Column: 125 × 4 5 μm Lichrospher RP-18
Mobile phase: MeOH:50 mM pH 1.9 phosphoric acid 30:70 containing 1 mM benzoic acid
Column temperature: 35
Flow rate: 0.9
Injection volume: 50
Detector: UV 273

CHROMATOGRAM
Retention time: 13.5

KEY WORDS
tablets; indirect UV detection

REFERENCE
Bechet,I.; Ceccato,A.; Hubert,P.; Herne,P.; Crommen,J. Determination of meprobamate in pharmaceutical dosage forms also containing carbromal by liquid chromatography and indirect photometric detection, *J.Pharm.Biomed.Anal.*, **1992**, *10*, 995–999.

Meptazinol



Molecular formula: $C_{15}H_{23}NO$
Molecular weight: 233.35
CAS Registry No.: 54340-58-8, 59263-76-2 (HCl)
Merck Index: 5910

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 20 μL 2.5 μg/mL IS in MeOH + 100 μL buffer + 3 mL ethyl acetate, vortex for 2 min, centrifuge at 2000 rpm for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 50°, reconstitute the residue in 100 μL MeOH, add 50 μL water, inject an aliquot. (Buffer was 1 M Na_2HPO_4 adjusted to pH 9.5 with 5 M NaOH.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb CN
Mobile phase: MeOH:0.5% ammonium acetate 45:55
Flow rate: 2
Detector: F ex 282 em 300

CHROMATOGRAM
Retention time: 5.8
Internal standard: m-(1-cyclopropylmethyl-3-ethylhexahydro-1H-azepin-3-yl)phenol (7.0)
Limit of detection: 3 ng/mL

KEY WORDS
plasma

REFERENCE
Frost,T. Determination of meptazinol in plasma by high-performance liquid chromatography with fluorescence detection, *Analyst*, **1981**, *106*, 999–1000.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Plasma + 100 μL 40 ng/mL fenethazine in 2 M aqueous Tris + 200 μL MTBE, vortex for 30 s, centrifuge at 9950 g for 2 min, inject a 100 μL aliquot of the organic phase.

HPLC VARIABLES
Column: 125 × 5 5 μm Spherisorb S5W silica
Mobile phase: MeOH:glacial acetic acid:ammonia 996:3:1 (Ammonia was 0.88 g/mL.)
Flow rate: 2
Injection volume: 100
Detector: E, EDT Research LCA 15, glassy carbon electrode +1.2 V, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 3
Internal standard: fenethazine (4)
Limit of detection: 500 ng/mL

KEY WORDS
plasma; cow; human

REFERENCE
Storey,G.C.A.; Schootstra,R.; Henry,J.A. Measurement of meptazinol in plasma by high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.*, **1985**, *341*, 223–227.

SAMPLE
Matrix: microsomal incubations, urine
Sample preparation: Microsomal incubations. 2 mL Microsomal incubation + IS + 1 mL 1 M pH 10.0 ammonia/ammonium chloride buffer + 5 mL n-hexane:ethyl acetate 50:50, extract. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 50 μL water, inject a 20 μL aliquot. Urine. Untreated or deconjugated (with 500-2000 IU/mL β-glucuronidase/sulfatase from Helix pomatia for 24 h) urine + IS + 1 mL 1 M pH 10.0 ammonia/ammonium chloride buffer + 5 mL n-hexane:ethyl acetate 30:70, stir vigorously for 10 min. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 50 μL water, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 30 × 4 10 μm LiChrospher 60 CN
Column: 250 × 4 5 μm LiChrospher 100 CN
Mobile phase: MeCN:MeOH:1% triethylammonium acetate buffer (pH 5.5) 15:5:80
Flow rate: 0.5
Injection volume: 20
Detector: UV 220

CHROMATOGRAM
Retention time: 31
Internal standard: 3-(3-ethylhexahydro-1-ethyl-1H-azepin-3-yl)phenol (35)
Limit of quantitation: 392 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
rabbit; liver; human; pharmacokinetics; rat

REFERENCE
Rudolphi,C.; Blaschke,G. Determination of the stereoselective aspects in in-vitro and in-vivo metabolism of the analgesic meptazinol by high-performance liquid chromatography, *J.Chromatogr.B*, **1995**, *663*, 315–326.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 3.6

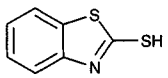
OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Mercaptobenzothiazole



Molecular formula: $C_7H_5NS_2$
Molecular weight: 167.26
CAS Registry No.: 149-30-4
Merck Index: 5916

SAMPLE

Matrix: blood, tissue, urine

Sample preparation: Urine. 1 mL Urine + 4 mL EtOH, centrifuge at 3000 rpm for 10 min. Dilute the supernatant with EtOH, filter (0.2 μm, Acro LC13), inject a 20 μL aliquot. (Hydrolyze conjugates by mixing 100 μL urine with 900 μL 100 mM pH 5 acetate buffer and 33 units sulfatase or 5000 units β-glucuronidase, heat at 37° for 30 min, proceed as above.) Tissue. Homogenize liver or kidney in 3 volumes of cold 100 mM pH 5 acetate buffer containing 2 mM aminooxyacetic acid, add 4 volumes of EtOH, centrifuge at 3000 rpm for 20 min. Filter (0.2 μm, Acro LC13) the supernatant and inject a 20 μL aliquot. Plasma. Add 4 volumes of EtOH to plasma, centrifuge at 3000 rpm for 20 min. Filter (0.2 μm, Acro LC13) the supernatant and inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 30 mm long C18
Column: 250 × 4.6 Partisil 10 ODS 3
Mobile phase: MeCN:water 50:50 adjusted to pH 4.5 with trifluoroacetic acid
Flow rate: 1
Injection volume: 20
Detector: UV 321

CHROMATOGRAM

Retention time: 7.0
Limit of detection: 200 nM

KEY WORDS

rat; hamster; guinea pig; mouse; plasma; liver; kidney

REFERENCE

Elfarra,A.A.; Hwang,I.Y. *In vivo* metabolites of *S*-(2-benzothiazolyl)-L-cysteine as markers of *in vivo* cysteine conjugate β-lyase and thiol glucuronosyl transferase activities, *Drug Metab.Dispos.*, **1990**, *18*, 917–922.

SAMPLE

Matrix: bulk, formulations

Sample preparation: Bulk. Weigh out 100 mg morphine, dissolve in 25 mL MeOH:water:acetic acid 24:72:1, dilute with MeOH:water:acetic acid 24:72:1 to a final concentration of 240 μg/mL morphine, filter (0.45 μm), inject a 20 μL aliquot. Injections. Dilute with MeOH:water:acetic acid 24:72:1 to a final concentration of 240 μg/mL morphine, filter (0.45 μm), inject a 20 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:5 mM sodium 1-heptanesulfonate:acetic acid 24:72:1
Flow rate: 1.5
Injection volume: 20
Detector: UV 230

CHROMATOGRAM

Retention time: 16

OTHER SUBSTANCES

Simultaneous: morphine (UV 284), phenol (UV 284), pseudomorphine (UV 230)

KEY WORDS
injections

REFERENCE
Bello,A.C.; Jhangiani,R.K. Liquid chromatographic determination of morphine sulfate and some contaminants in injections and bulk drug material: collaborative study, *J.Assoc.Off.Anal.Chem.*, **1988**, *71*, 1046–1048.

SAMPLE
Matrix: formulations
Sample preparation: Directly inject a 20 μL aliquot of a 250 μg/mL digoxin injection.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:water 29:71
Flow rate: 2
Injection volume: 20
Detector: UV 218

CHROMATOGRAM
Retention time: 7

OTHER SUBSTANCES
Simultaneous: digoxin

KEY WORDS
injections

REFERENCE
Reepmeyer,J.C.; Juhl,Y.H. Contamination of injectable solutions with 2-mercaptobenzothiazole leached from rubber closures, *J.Pharm.Sci.*, **1983**, *72*, 1302–1305.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 100 × 3 5 μm ChromSpher C18 (Chrompack)
Mobile phase: MeOH:water:acetic acid 45:54:1
Flow rate: 0.4
Injection volume: 75
Detector: UV 321

CHROMATOGRAM
Retention time: 5.5
Limit of detection: 5 μM

REFERENCE
Stijntjes,G.J.; te Koppele,J.M.; Vermeulen,N.P.E. High-performance liquid chromatography-fluorescence assay of pyruvic acid to determine cysteine conjugate β-lyase activity: application to *S*-1,2-dichlorovinyl-L-cysteine and *S*-2-benzothiazolyl-L-cysteine, *Anal.Biochem.*, **1992**, *206*, 334–343.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in MeCN, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm Spheri-10 RP-18
Mobile phase: MeCN:water:acetic acid 30:70:0.03
Flow rate: 1
Injection volume: 50

Detector: UV 328

CHROMATOGRAM
Retention time: 2
Limit of detection: 500 ng

REFERENCE
Gaind,V.S.; Jedrzejczak,K. HPLC determination of rubber septum contaminants in the iodinated intravenous contrast agent (sodium iothalamate), *J.Anal.Toxicol.*, **1993**, *17*, 34–37.

SAMPLE
Matrix: solutions
Sample preparation: Mix 500 μL of a solution in MeOH:water 90:10 with 500 μL 50 μM CY5.4a-IA in MeOH:water 70:30, pass dry nitrogen through the mixture for 1 min, heat at 65° for 1.5 h, inject a 25 μL aliquot. (Some details for the synthesis of CY5.4a-IA are given in the paper.)

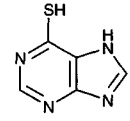
HPLC VARIABLES
Column: 150 × 3.1 5 μm LiChrosorb RP-8
Mobile phase: MeOH:10 mM pH 6.8 phosphate buffer 65:35 containing 1 mM triethylamine
Flow rate: 0.75
Injection volume: 25
Detector: F ex 670 (9.5 mW Lasermax LAS200-670-10 diode laser)

CHROMATOGRAM
Retention time: 7
Limit of detection: 1 nM

KEY WORDS
derivatization

REFERENCE
Mank,A.J.G.; Molenaar,E.J.; Lingeman,H.; Gooijer,C.; Brinkman,U.A.T.; Velthorst,N.H. Visible diode laser induced fluorescence detection in liquid chromatography after precolumn derivatization of thiols, *Anal.Chem.*, **1993**, *65*, 2197–2203.

Mercaptopurine



Molecular formula: $C_5H_4N_4S$
Molecular weight: 152.18
CAS Registry No.: 50-44-2, 6112-76-1 (monohydrate)
Merck Index: 5919

SAMPLE
Matrix: blood
Sample preparation: Condition a 500 mg Bakerbond SPE cartridge packed with LiChrosorb RP-18 with 2 mL MeOH, 2 mL water and 1 mL MeOH. Make up 500 μL plasma to 3 mL with MeOH. Centrifuge at 1100 g for 15 min, add 1.5 mL supernatant to the SPE cartridge, elute at 50 μL/min flow-rate, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 200 × 4 7 μm Lichrosorb RP-18
Mobile phase: MeOH: pH 4.15 phosphate buffer 20:80
Flow rate: 1
Injection volume: 20
Detector: UV 262

CHROMATOGRAM
Retention time: 3.30
Internal standard: mercaptopurine

KEY WORDS
plasma; SPE; mercaptopurine is IS

REFERENCE
Misztal,G.; Paw,B. Determination of fludarabine phosphate in human plasma using reversed phase high-performance liquid chromatography, *Pharmazie*, **1996**, *51*, 733–734.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Blood + 120 μg dithiothreitol (to prevent oxidation of 6-mercaptopurine), centrifuge at 2000 g for 5 min. Cool plasma in ice, add a volume of ice-cold 50% trichloroacetic acid equal to 10% of the plasma volume, mix vigorously, keep on ice for 10 min, centrifuge at 2000 g for 10 min. Remove the supernatant and adjust the pH to 6-7 with 4 M KOH, inject a 460 μL aliquot.

HPLC VARIABLES
Column: Two 250 × 4.6 10 μm Spherisorb 10-ODS columns in series
Mobile phase: 50 mM pH 6.35 potassium phosphate buffer
Flow rate: 1.5
Injection volume: 460
Detector: UV 312

CHROMATOGRAM
Retention time: 9
Limit of detection: 3 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; pharmacokinetics; dog

REFERENCE
De Abreu,R.A.; van Baal,J.M.; Schouten,T.J.; Schretlen,E.D.A.M.; de Bruyn,C.H.M.M. High-performance liquid chromatographic determination of plasma 6-mercaptopurine in clinically relevant concentrations, *J.Chromatogr.*, **1982**, *227*, 526–533.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 25 μL 4 μg/mL 6-thioguanine in water + 100 μL 400 mM NaOH + 1 mL 0.3% phenylmercuric acetate in ethyl acetate + 3 mL diethyl ether, shake on a tumble mixer for 10 min, centrifuge for 5 min. Remove the organic layer and add it to 500 μL 100 mM HCl, whirlmix for 2 min, centrifuge for 5 min, discard the organic layer, evaporate traces of organic solvent under a stream of nitrogen at room temperature for 15 min, add 10 μL 3 mg/mL dithioerythritol in water, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 LiChrosorb 10 RP-18
Mobile phase: Isopropanol:water 3:97 containing 13.80 g/L $NaH_2PO_4.H_2O$, 200 μL/L 85% phosphoric acid, 60 mg/L dithioerythritol, and 500 mg/L sodium octanesulfonate, pH 3.6-3.7
Flow rate: 1.5
Detector: F ex 295 em 380 following post-column reaction. The column effluent mixed with 8 mM potassium chromate in 500 mM HCl pumped at 0.16 mL/min and with air flowing at 0.32 mL/min and the mixture flowed through a single mixing coil. The effluent from this coil mixed with 1.6% sodium metabisulfite pumped at 0.16 mL/min and this mixture flowed through a single mixing coil. The effluent from this coil mixed with 4 M ammonium hydroxide pumped

at 0.23 mL/min and this mixture flowed through a double mixing coil to a debubbler. The liquid effluent from the debubbler flowed to the detector.

CHROMATOGRAM
Retention time: 6
Internal standard: 6-thioguanine (8)
Limit of detection: <2 ng/mL

KEY WORDS
post-column reaction; plasma; pharmacokinetics

REFERENCE
Jonkers,R.E.; Oosterhuis,B.; ten Berge,R.J.M.; van Boxtel,C.J. Analysis of 6-mercaptopurine in human plasma with a high-performance liquid chromatographic method including post-column derivatization and fluorimetric detection, *J.Chromatogr.*, **1982**, *233*, 249–255.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 80 μL 10 μg/mL 6-thioguanine in water + 10 μL 1 M dithiothreitol, vortex for 10 s, add 2 mL MeCN, vortex for 30 s, centrifuge at 2000 g for 5 min. Remove the supernatant and add it to 2 mL dichloromethane, shake on a reciprocating shaker for 5 min, centrifuge at 2000 g for 5 min. Remove 750 μL from the top aqueous layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 150 μL distilled water, vortex for 1 min, inject a 15 μL aliquot.

HPLC VARIABLES
Guard column: 70 × 2.2 30-38 μm Co:Pell ODS
Column: 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase: MeCN:acetic acid:water 3.5:0.2:96.3
Flow rate: 1.4
Injection volume: 15
Detector: UV 322

CHROMATOGRAM
Retention time: 4.8
Internal standard: 6-thioguanine (6.5)
Limit of detection: 5 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Noninterfering: caffeine, cytarabine, 5-fluorouracil, prednisone, theophylline, vinblastine, vincristine

KEY WORDS
plasma; protect from light; monkey; pharmacokinetics; human

REFERENCE
Narang,P.K.; Yeager,R.L.; Chatterji,D.C. Quantitation of 6-mercaptopurine in biologic fluids using high-performance liquid chromatography: a selective and novel procedure, *J.Chromatogr.*, **1982**, *230*, 373–380.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Serum + 5 μL 50 μg/mL 2-ethyl-4-oxoquinazoline in EtOH + 100 μL reagent, let stand at room temperature for 1 h, add 1.8 mL ethyl acetate, mix, centrifuge at 1800 g for 5 min, remove 1.5 mL of the supernatant, repeat the extraction. Combine the organic layers and evaporate them to dryness under reduced pressure below 30°, reconstitute the residue in 100 μL initial mobile phase, inject a 90 μL aliquot. (Reagent was 30 mg N-ethylmaleimide in 2 mL 50 mM pH 7.0 phosphate buffer, prepare fresh daily.)

HPLC VARIABLES
Column: 10 μm μBondapak C18

Mobile phase: Gradient. MeCN:10 mM KH_2PO_4 9:91 for 26 min, then 50:50 for 1 min (step gradient).
Flow rate: 1.5
Injection volume: 90
Detector: UV 280

CHROMATOGRAM
Retention time: 18.6
Internal standard: 2-ethyl-4-oxoquinazoline (28)
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES
Extracted: azathioprine

KEY WORDS
serum; derivatization

REFERENCE
Tsutsumi,K.; Otsuki,Y.; Kinoshita,T. Simultaneous determination of azathioprine and 6-mercaptopurine in serum by reversed-phase high-performance liquid chromatography, *J.Chromatogr.*, **1982**, *231*, 393–399.

SAMPLE
Matrix: blood
Sample preparation: 250 μL Plasma + 5 μL 2.5 μg/mL 6-thioguanine + 25 μL 1 M aluminum perchlorate in water, let stand at room temperature for 15 min, chill in ice water for 15 min, centrifuge at 15600 g for 15 min, discard the supernatant. Suspend the precipitate in 500 μL 50 mM aluminum perchlorate in water by stirring to break up the precipitate, vortex for 20 s, centrifuge at 15600 g for 15 min, discard the supernatant. Suspend the precipitate in 150 μL 400 mM perchloric acid, add 5 μL freshly prepared 200 mM aqueous sodium hydrosulfite, mix, let stand at room temperature for 30 min, chill in ice water, centrifuge at 15600 g for 15 min, inject a 50 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: 45 × 2 40 μm ODS
Column: 150 × 4.3 5 μm Ultrasphere ODS
Mobile phase: Water:85% phosphoric acid 99.32:0.68 containing 154.3 mg/L dithiothreitol
Flow rate: 1
Injection volume: 50
Detector: UV 340

CHROMATOGRAM
Retention time: 6.08
Internal standard: 6-thioguanine (4.73)
Limit of quantitation: 3 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, 6-thiouric acid

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Lin,K.T.; Varin,F.; Rivard,G.E.; Leclerc,J.M. Isolation of 6-mercaptopurine in human plasma by aluminum ion complexation for high-performance liquid chromatographic analysis, *J.Chromatogr.*, **1991**, *536*, 349–355.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Plasma + 800 μL MeOH, stir for 15 s, let stand for 20 min, centrifuge at 5° at 3000 rpm for 10 min. Remove 800 μL of the supernatant and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μL mobile phase, inject a 40 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 ODS-II (Shimadzu)
Mobile phase: MeOH:water 7:100 containing 0.2% glacial acetic acid and 0.1% sodium 1-heptanesulfonate
Flow rate: 1
Injection volume: 40
Detector: UV 325

CHROMATOGRAM
Retention time: 6.8
Limit of detection: 2 ng/mL

KEY WORDS
plasma; pharmacokinetics; rat

REFERENCE
Takeichi,Y.; Kimura,T. Improvement of aqueous solubility and rectal absorption of 6-mercaptopurine by addition of sodium benzoate, *Biol.Pharm.Bull.*, **1994**, *17*, 1391–1394.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 50 μL 2.8 μg/mL 6-mercaptopurine arabinoside + 2-3 mg mercurial cellulose, vortex for 30 s, centrifuge at 550 g for 5 min. Resuspend the pellet in 2 mL phosphate buffered saline, centrifuge, repeat this process twice. Suspend the pellet in 250 μL 20 mM 2-mercaptoethanol, centrifuge, inject a 100 μL aliquot of the supernatant. (Prepare mercurial cellulose as follows. Add 40 g Sigmacell Type 20 cellulose (20 μm) to 175 mL 40% NaOH at 5°, let stand at 0° for 2 h, add 40 mL allyl glycidyl ether dropwise, add 100 mL water, heat at 70-80° for 2 h. Suspend the cellulose in 4 L water, allow to settle, decant the water, wash several more times, filter, wash with water until the pH of the filtrate is about 8, wash with 1 L 10% acetic acid, wash 1 L with EtOH:water 95:5, wash with 1 L MeOH, wash with 1 L diethyl ether, air dry. Add to 400 mL 10% acetic acid containing 2.3 g mercuric acetate, stir at 60° for 1 h, filter, wash with 2 L 10% acetic acid, wash with 1.5 L water, wash with 300 mL EtOH:water 95:5, wash with 700 mL MeOH, wash with 1 L diethyl ether, air dry in the dark, store in the dark (Anal.Biochem. 1985, 144, 514).)

HPLC VARIABLES
Column: 250 × 4.6 5 μm ODS (Beckman)
Mobile phase: MeCN:10 mM pH 3.0 sodium phosphate buffer 2:98
Flow rate: 1.2
Injection volume: 100
Detector: UV 323

CHROMATOGRAM
Retention time: 7.6
Internal standard: 6-mercaptopurine arabinoside (13.6)
Limit of detection: 0.5 ng/mL

OTHER SUBSTANCES
Noninterfering: aspirin, chloroquine, cyclosporin, diltiazem, nifedipine, prednisolone

KEY WORDS
plasma; pharmacokinetics; SPE

REFERENCE
Albertioni,F.; Pettersson,B.; Ohlman,S.; Peterson,C. Analysis of azathioprine and 6-mercaptopurine in plasma in renal transplant recipients after administration with oral azathioprine, *J.Liq.Chromatogr.*, **1995**, *18*, 3991–4005.

SAMPLE
Matrix: blood

Sample preparation: Add 1 volume ice-cold 8 M perchloric acid to 20 volumes plasma, mix, keep on ice for 10 min, centrifuge at 10 000 g for 15 min, remove the supernatant. Adjust the pH of the supernatant to 6-7 with 10 volumes ice-cold 4 M K_2KHPO_4, keep on ice for 10 min, centrifuge at 10 000 g for 5 min, inject a 100 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-18-DB
Mobile phase: Gradient. A was 25 mM KH_2PO_4. B was MeOH:50 mM KH_2PO_4 25:75. A:B from 98:2 to 96:4 over 5 min, to 85:15 over 3 min, to 80:20 over 2 min, to 40:60 over 10 min, maintain at 40:60 over 2 min, to 20:80 over 3 min, maintain at 20:80 for 20 min, return to initial conditions over 3 min, re-equilibrate for 12 min.
Flow rate: 1.25
Injection volume: 100
Detector: UV 320

CHROMATOGRAM
Retention time: 11.6
Limit of detection: 20-50 nM

OTHER SUBSTANCES
Extracted: metabolites, thioguanine (UV 342)

KEY WORDS
plasma

REFERENCE
Keuzenkamp-Jansen,S.W.; De Abreu,R.A.; Bökkerink,J.P.M.; Trijbels,J.M.F. Determination of extracellular and intracellular thiopurines and methylthiopurines by high-performance liquid chromatography, *J.Chromatogr.B*, **1995**, *672*, 53–61.

SAMPLE
Matrix: blood
Sample preparation: Treat whole blood with EDTA, freeze a 2 mL aliquot at -20°, thaw, add 2 mL PBS, centrifuge at 3000 g for 15 min, suspend the pellet in 200 μL PBS, treat with proteinase K at 70° for 10 min, add to a disposable spin column (Diagen QIAamp Blood Kit, Hilden, Germany), centrifuge for 1 min, wash twice with buffer for 1 min, elute with 200 μL 10 mM pH 9.0 Tris-HCl buffer containing 0.1 mM EDTA, heat at 100° for 5 min, cool in ice. Remove a 100 μl aliquot and add it to 10 μL buffer, add 20 μL 25 μg/mL P_1 nuclease (Boehringer Mannheim) and 12.5 U/mL acid phosphatase (Sigma) in buffer:water 10:90, heat at 42° for 1 h, add 10 μL 400 mM formic acid, add 60 μL MeOH, add 1 μL 5 mM N-[6-(7-amino-4-methylcoumarin-3-acetamido)hexyl]-3'-(2'-pyridyldithio)propionamide (AMCA-HPDP, Pierce) in DMF, inject a 25 μL aliquot. (Buffer was 500 mM pH 4.5 sodium acetate buffer containing 10 mM magnesium chloride.)

HPLC VARIABLES
Guard column: 20 mm long Supelguard (Supelco)
Column: 150 × 4.6 3 μm Supelcosil LC-8
Mobile phase: MeOH:buffer 37:63 (Between analyses wash column with MeOH:buffer 80:20 for 3 min. Buffer was 200 mM formic acid adjusted to pH 4.0 with 10 M NaOH.)
Column temperature: 45
Flow rate: 1
Injection volume: 25
Detector: F ex 345 em 450

CHROMATOGRAM
Retention time: 11.5 (as 2'-deoxy-6-thioguanosine metabolite)
Limit of detection: 60 pmole/g DNA

KEY WORDS
derivatization; whole blood

REFERENCE

Warren,D.J.; Andersen,A.; Slordal,L. Quantitation of 6-thioguanine residues in peripheral blood leukocyte DNA obtained from patients receiving 6-mercaptopurine-based maintenance therapy, *Cancer Res.*, **1995**, *55*, 1670–1674.

SAMPLE

Matrix: blood, CSF, urine

Sample preparation: Blood, CSF. Collect 2 mL blood in tubes containing heparin and 120 μg dithiothreitol (DTT). Mix, centrifuge at 2000 g for 5 min, remove plasma. CSF. Collect 0.5 mL CSF in tubes containing 30 μg DTT. Cool CSF and plasma samples on ice, add freshly prepared ice-cold 50% trichloroacetic acid equal to 10% of sample volume. Urine. Collect 2 mL urine in tubes containing 120 μg DTT, filter (0.22 μm), inject an aliquot.

HPLC VARIABLES

Column: two 250 × 4.6 10μm Nucleosil 10 C18 columns in series

Mobile phase: Gradient. A was 25 mM pH 2.75 phosphoric acid. B was MeOH:water 50:50. C was 100 mM pH 6.6 KH_2PO_4. A:B:C from 100:0:0 to 98:2:0 over 5 min, to 30:3.5:66.5 over 5 min, maintain at 30:3.5:66.5 for 10 min, re-equilibrate at initial conditions for 10 min.

Column temperature: 33

Flow rate: 1.7

Injection volume: 195, 500

Detector: UV 312

CHROMATOGRAM

Retention time: 14

Limit of detection: 20 nM

OTHER SUBSTANCES

Extracted: metabolites, 6-mercaptopurine riboside, thioguanine (UV 342), 6-thioguanosine

KEY WORDS

plasma; goat; pharmacokinetics

REFERENCE

van Baal,J.M.; van Leeuwen,M.B.; Schouten,T.J.; De Abreu,R.A. Sensitive high-performance liquid chromatographic determination of 6-mercaptopurine, 6-thioguanine, 6-mercaptopurine riboside and 6-thioguanosine in biological fluids, *J.Chromatogr.*, **1984**, *336*, 422–428.

SAMPLE

Matrix: enzyme incubations

Sample preparation: To 237 μL enzyme incubation add 850 μL ice-cold 3.5 mM DL-dithiothreitol immediately followed by 500 μL 1.5 M sulfuric acid, place tubes on ice. Equilibrate tubes to room temperature, heat at 100° for 2 h. Cool, add 500 μL 3.4 M NaOH immediately followed by 8 mL toluene:amyl alcohol:phenyl mercury acetate mixture. Shake gently for 10 min, centrifuge at 10° at 900 g for 5 min. Transfer 6 mL toluene to another tube and add 200 μL 100 mM HCl. Vortex for four 20 s periods, centrifuge at 10° at 900 g for 5 min, inject a 50 μL aliquot of the aqueous layer. (Prepare toluene:amyl alcohol:phenyl mercury acetate mixture by adding enough phenyl mercury acetate to toluene containing 170 mM amyl alcohol so as to form a solution containing 1.3 mM phenyl mercury acetate, mix gently for 1 h, store in the dark (J.Chromatogr. 1992, 583, 83).)

HPLC VARIABLES

Guard column: 5 × 4 5 μm Resolve C18

Column: 100 × 8 5μm Resolve C18 radial compression

Mobile phase: MeOH:water 20:80 containing 100 mM triethylamine and 0.5 mM dithiothreitol, pH adjusted to 3.2 with orthophosphoric acid (Add dithiothreitol immediately prior to use.)

Flow rate: 1.5

Injection volume: 50

Detector: UV 303

CHROMATOGRAM

Retention time: 4

OTHER SUBSTANCES
Extracted: methylmercaptopurine

REFERENCE
Lennard,L.; Singleton,H.J. High-performance liquid chromatographic assay of human red blood cell thiopurine methyltransferase activity, *J.Chromatogr.B*, **1994**, *661*, 25–33.

SAMPLE
Matrix: formulations
Sample preparation: Dissolve crushed tablets or the freeze-dried compound for injection in 20 mM NaOH. Add a 10 mL aliquot of this solution (or a saline injection) to 10 mL 3 mg/mL theophylline in 20 mM NaOH, inject a 1.5 μL aliquot.

HPLC VARIABLES
Column: 100 × 5 5 μm ODS-Hypersil
Mobile phase: MeOH:25 mM KH_2PO_4:glacial acetic acid 20:79:5 adjusted to pH 4.50 (Flush column with MeOH:water 60:40 at the end of each day.)
Flow rate: 1.5
Injection volume: 1.5
Detector: UV 240

CHROMATOGRAM
Retention time: 1.7
Internal standard: theophylline (3.5)

OTHER SUBSTANCES
Simultaneous: azathioprine, impurities, degradation products

KEY WORDS
stability-indicating; injections; tablets

REFERENCE
Fell,A.F.; Plag,S.M.; Neil,J.M. Stability-indicating assay for azathioprine and 6-mercaptopurine by reversed-phase high-performance liquid chromatography, *J.Chromatogr.*, **1979**, *186*, 691–704.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 25 μL aliquot.

HPLC VARIABLES
Guard column: 40 × 4 C18 Corasil II
Column: 300 × 4 10 μm μBondapak phenyl
Mobile phase: Propanol:6 mM C_{12} DAPS (Fluka) 3:97 (C_{12} DAPS is 3-(dimethyldodecylammonio)propanesulfonate.)
Injection volume: 25
Detector: UV 273

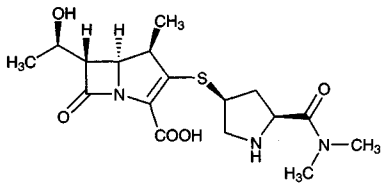
CHROMATOGRAM
Retention time: 3.3

OTHER SUBSTANCES
Simultaneous: albendazole, aminophylline, antipyrine, caffeine, dipropyline, flubendazole, metronidazole, nimorazole, procaine, β-hydroxytheophylline, theophylline, tinidazole
Interfering: albendazole sulfoxide, amyleine, theobromine

KEY WORDS
micellar chromatography

REFERENCE
Habel,D.; Guermouche,S.; Guermouche,M.H. Direct determination of theophylline in human serum by high-performance liquid chromatography using zwitterionic micellar mobile phase. Comparison with an enzyme multiplied immunoassay technique, *Analyst*, **1993**, *118*, 1511–1513.

Meropenem



Molecular formula: $C_{17}H_{25}N_3O_5S$
Molecular weight: 383.45
CAS Registry No.: 96036-03-2
Merck Index: 5960

SAMPLE
Matrix: bile, blood
Sample preparation: Condition a 100 mg Bond Elut C8 SPE cartridge with two 1 mL portions of MeOH and two 1 mL portions of 500 mM KH_2PO_4. 100 µL Plasma or 50 µL bile + 900 µL water, mix, add to the SPE cartridge, wash with 1 mL 50 mM KH_2PO_4, elute with 800 (plasma) or 600 (bile) µL MeOH:50 mM pH 6.0 phosphate buffer 10:90, inject a 100 µL aliquot of the eluate.

HPLC VARIABLES
Column: 100 × 4.6 3 µm Hypersil ODS
Mobile phase: MeOH:buffer 15:85 (Buffer was 50 (plasma) or 57.5 (bile) mM pH 7.0 phosphate buffer.)
Flow rate: 1
Injection volume: 100
Detector: UV 296 (plasma), UV 310 (bile)

CHROMATOGRAM
Retention time: 5-5.5
Limit of detection: 280 ng/mL (bile), 100 ng/mL (plasma)

KEY WORDS
plasma; SPE

REFERENCE
Granai,F.; Smart,H.L.; Triger,D.R. A study of the penetration of meropenem into bile using endoscopic retrograde cholangiography, *J.Antimicrob.Chemother.*, **1992**, *29*, 711–718.

SAMPLE
Matrix: blood
Sample preparation: Dilute 50 µL plasma with 75 µL 50mM pH 7.0 phosphate buffer, inject a 100 µL aliquot onto column A with mobile phase A, elute to waste with mobile phase A, after 4 min backflush the contents of column A onto column B with mobile phase B, monitor the effluent from column B. After 11 min re-equilibrate column A with mobile phase A and column B with mobile phase B for 5 min.

HPLC VARIABLES
Column: 20 × 3.9 25-40 µm LiChroprep RP-8; B 4.0 × 10 Nova -Pak C8 + 150 × 4.6 5 µm Inertsil ODS
Mobile phase: A 50 mM pH 7.0 phosphate buffer; B MeCN:50 mM pH 7.0 phosphate buffer 6:94
Flow rate: 1
Injection volume: 100
Detector: UV 300

CHROMATOGRAM
Retention time: 6.5

Limit of detection: 100 ng/mL

KEY WORDS
rat; plasma; column-switching; pharmacokinetics

REFERENCE
Lee,H.S.; Shim,H.O.; Yu,S.R. High-performance liquid chromatographic determination of meropenem in rat plasma using column-switching, *Chromatographia*, **1996**, *42*, 405–408.

SAMPLE
Matrix: blood
Sample preparation: Dilute serum with an equal volume of water, inject 20 μL diluted serum onto column A, elute to waste with mobile phase A, after 1.1 min elute the contents of column A to column B with mobile phase B, elute with mobile phase B, monitor the effluent from column B.

HPLC VARIABLES
Column: A 50 × 2.1 40 μm Supelclean LC-NH_2 (Supelco); B 150 × 4.6 3 μm C18 (Supelco)
Mobile phase: A MeOH:10 mM pH 7.0 phosphate buffer 5:95; B MeOH:10 mM pH 7.0 phosphate buffer containing 5 mM tetrabutylammonium hydrogen sulfate 30:70
Flow rate: A 0.3; B 1
Injection volume: 20
Detector: UV 298

CHROMATOGRAM
Retention time: 4.2
Limit of detection: 100 ng/mL

OTHER SUBSTANCES
Noninterfering: acetaminophen, acyclovir, digoxin, fluconazole, theophylline, vancomycin

KEY WORDS
column-switching; serum

REFERENCE
Bompadre,S.; Ferrante,L.; de Martinis,M.; Leone,L. Determination of meropenem in serum by high-performance liquid chromatography with column switching, *J.Chromatogr.A*, **1998**, *812*, 249–253.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Plasma + 900 μL water, add to a conditioned Bond Elut C18 SPE cartridge, wash with 1 mL 50 mM KH_2PO_4, elute with 800 μL mobile phase, inject an aliquot of the eluate.

HPLC VARIABLES
Column: 100 mm long 3 μm Hypersil ODS
Mobile phase: MeOH:5 mM tetrabutylammonium dihydrogen phosphate 12:88
Flow rate: 1
Detector: UV 296

CHROMATOGRAM
Retention time: 9
Limit of detection: 200 ng/mL

KEY WORDS
SPE; plasma; pharmacokinetics

REFERENCE
Harrison,M.P.; Haworth,S.J.; Moss,S.R.; Wilkinson,D.M.; Featherstone,A. The disposition and metabolic fate of ^{14}C-meropenem in man, *Xenobiotica*, **1993**, *23*, 1311–1323.

SAMPLE
Matrix: blood
Sample preparation: Dilute plasma with water, prepare using a 1 mL C18 SPE cartridge.

HPLC VARIABLES
Column: 100 × 4.6 3 μm Hypersil C18
Mobile phase: MeOH:5 mM tetrabutylammonium dihydrogen phosphate 15:85
Detector: UV 296

CHROMATOGRAM
Limit of quantitation: 500 ng/mL

KEY WORDS
plasma; SPE; pharmacokinetics

REFERENCE
Bedikian,A.; Okamoto,M.P.; Nakahiro,R.K.; Farino,J.; Heseltine,P.N.R.; Appleman,M.D.; Yellin,A.E.; Berne,T.V.; Gill,M.A. Pharmacokinetics of meropenem in patients with intra-abdominal infections, *Antimicrob.Agents Chemother.*, **1994**, *38*, 151–154.

SAMPLE
Matrix: blood, formulations, urine
Sample preparation: Dilute plasma 1:4, urine 1:10, and injections 1:100 with water, filter (0.6 μm), inject a 5-50 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 200 × 4 5 μm Nucleosil C18
Mobile phase: MeOH:10 mM pH 7.4 potassium phosphate buffer 18:82 (plasma, injections) or 25:75 (urine)
Flow rate: 1
Injection volume: 5-50
Detector: UV 296

CHROMATOGRAM
Limit of detection: 5 μg/mL (urine), 500 ng/mL (plasma)

KEY WORDS
plasma; injections; saline; pharmacokinetics

REFERENCE
Burman,L.Å.; Nilsson-Ehle,I.; Hutchison,M.; Haworth,S.J.; Norrby,S.R. Pharmacokinetics of meropenem and its metabolite ICI 213,689 in healthy subjects with known renal metabolism of imipenem, *J.Antimicrob.Chemother.*, **1991**, *27*, 219–224.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. Condition a Bond Elut C18 SPE cartridge. Add 100 μL plasma to the SPE cartridge, wash with 50 mM KH_2PO_4, elute with 800 μL eluent, inject an aliquot of the eluate. Urine. Dilute urine 1:10 with water, inject an aliquot directly. (Eluent was MeOH: 5 mM tetrabutylammonium dihydrogen phosphate 12:88.)

HPLC VARIABLES
Column: 100 × 4 3 μm Hypersil ODS
Mobile phase: MeOH:5 mM tetrabutylammonium dihydrogen phosphate 12:88 (plasma) or MeCN:10 mM pH 7.4 phosphate buffer 6:100 (urine)
Detector: UV 296

CHROMATOGRAM
Limit of detection: 60 ng/mL (plasma), 10 μg/mL (urine)

KEY WORDS
SPE; plasma; pharmacokinetics

REFERENCE
Bax,R.P.; Bastain,W.; Featherstone,A.; Wilkinson,D.M.; Hutchison,M. Haworth,S.J. The pharmacokinetics of meropenem in volunteers, *J.Antimicrob.Chemother.*, **1989**, *24*, 311–320.

SAMPLE
Matrix: enzyme incubations
Sample preparation: Add 2 volumes of MeOH, mix well, centrifuge at 3000 g for 15 min, inject a 10 μL aliquot of the supernatant.

HPLC VARIABLES
Column: Inertsil ODS-2
Mobile phase: MeOH:5 mM tetrabutylammonium phosphate 30:70
Flow rate: 1
Injection volume: 10
Detector: UV 300

CHROMATOGRAM
Limit of quantitation: 1 μg/mL

REFERENCE
Hikida,M.; Kawashima,K.; Yoshida,M.; Mitsuhashi,S. Inactivation of new carbapenem antibiotics by dehydropeptidase-I from porcine and human renal cortex, *J.Antimicrob.Chemother.*, **1992**, *30*, 129–134.

SAMPLE
Matrix: urine
Sample preparation: Dilute urine 1:5 with water, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 50 mm long 3 μm Hypersil ODS
Mobile phase: MeCN:10 mM pH 7.4 phosphate buffer 6:100
Flow rate: 1
Injection volume: 50
Detector: UV 296

CHROMATOGRAM
Retention time: 5.5
Limit of detection: 600 ng/mL

KEY WORDS
pharmacokinetics

REFERENCE
Harrison,M.P.; Haworth,S.J.; Moss,S.R.; Wilkinson,D.M.; Featherstone,A. The disposition and metabolic fate of ^{14}C-meropenem in man, *Xenobiotica*, **1993**, *23*, 1311–1323.

SAMPLE
Matrix: urine
Sample preparation: Dilute urine with water, prepare using a 1 mL C18 SPE cartridge.

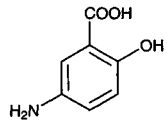
HPLC VARIABLES
Column: 150 × 4.6 5 μm Partisil silica
Mobile phase: 0.1% Phosphoric acid in water
Detector: UV 313

CHROMATOGRAM
Limit of quantitation: 1 μg/mL

KEY WORDS
SPE; pharmacokinetics

REFERENCE
Bedikian,A.; Okamoto,M.P.; Nakahiro,R.K.; Farino,J.; Heseltine,P.N.R.; Appleman,M.D.; Yellin,A.E.; Berne,T.V.; Gill,M.A. Pharmacokinetics of meropenem in patients with intra-abdominal infections, *Antimicrob.Agents Chemother.*, **1994**, *38*, 151–154.

Mesalamine



Molecular formula: $C_7H_7NO_3$
Molecular weight: 153.14
CAS Registry No.: 89-57-6
Merck Index: 5964

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 4 mL MeOH, let stand for 15 min at 4°, centrifuge at 3000 g for 15 min. Remove 1 mL of the supernatant and add it to 1 mL water, mix, inject an aliquot.

HPLC VARIABLES
Column: 40 × 4.6 3 μm Hypersil
Mobile phase: MeOH:water:200 mM pH 6.5 potassium phosphate buffer 40:40:20 containing 4 mM hexadecyltrimethylammonium bromide
Column temperature: 34
Flow rate: 1.7
Injection volume: 20
Detector: F ex 315 em 470

CHROMATOGRAM
Retention time: 2.5

KEY WORDS
plasma

REFERENCE
Tjornelund,J.; Hansen,S.H. Stability of 5-aminosalicylic acid and its metabolites in plasma at -20°C. Formation of N-β-D-glucopyranosyl-5-aminosalicylic acid, *J.Chromatogr.*, **1991**, *570*, 224–228.

SAMPLE
Matrix: blood, feces, urine
Sample preparation: Plasma. 1 mL Plasma + 2 mL MeOH, vortex for 20 s, let stand for 15 min at -20° and 10 min at room temperature, mix, centrifuge at 2000 g for 15 min. Remove the supernatant and add it to 5 mL 1,1,1-trichloroethane, shake for 2 min, centrifuge at 2000 g for 15 min, inject a 20 μL aliquot of the supernatant. Urine. 1 mL Urine + 4 mL MeOH, mix, let stand at -20° for 15 min, centrifuge at 2000 g for 15 min. Dilute an aliquot of the supernatant with an equal volume of water, inject a 20 μL aliquot. Feces. Extract with 500-1000 mL MeOH, centrifuge at 10000 g for 4 min. Dilute an aliquot of the supernatant with an equal volume of water, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 40 × 4.6 3 μm Hypersil
Mobile phase: MeOH:water:200 mM pH 6.5 potassium phosphate buffer 40:40:20 containing 4 mM hexadecyltrimethylammonium bromide
Column temperature: 34
Flow rate: 1.7
Injection volume: 20

Detector: F ex 315 em 470

CHROMATOGRAM
Retention time: 2.5
Limit of detection: 4 ng/mL

OTHER SUBSTANCES
Simultaneous: metabolites

KEY WORDS
plasma

REFERENCE
Tjornelund,J.; Hansen,S.H. High-performance liquid chromatographic assay of 5-aminosalicylic acid (5-ASA) and its metabolites N-β-D-glucopyranosyl-5-ASA, N-acetyl-5-ASA, N-formyl-5-ASA and N-butyryl-5-ASA in biological fluids, *J.Chromatogr.*, **1991**, *570*, 109–117.

SAMPLE
Matrix: blood, urine
Sample preparation: Serum. Add disodium-3,5'-azo-bis-(6-hydroxybenzoate) to serum, treat with proteinase K (0.5 mg/mL protein) for 10 min, add tetrabutylammonium hydrogen sulfate buffered to pH 6.5, add dichloromethane, agitate for 30 min, centrifuge. Remove the organic layer and evaporate it to dryness, reconstitute the residue in mobile phase, inject an aliquot. Urine. Add 2,4-dihydroxybenzoic acid to urine, react with propionic anhydride for 5 min, add perchloric acid, add diethyl ether, shake for 10 min, freeze. Remove the organic layer and add it to pH 7.4 phosphate buffer, extract, inject an aliquot of the aqueous phase.

HPLC VARIABLES
Guard column: 30 × 4 30-40 μm Perisorb RP-18
Column: 250 × 4 10 μm Nucleosil C18
Mobile phase: MeOH:buffer 30:70 (Buffer was pH 7.4 phosphate buffer containing 20 mM tetrabutylammonium hydrogen sulfate.)
Detector: F ex 312 em 469

CHROMATOGRAM
Retention time: k' 2.4 (propionyl derivative)
Internal standard: disodium-3,5'-azo-bis-(6-hydroxybenzoate), 2,4-dihydroxybenzoic acid
Limit of quantitation: 6.1 μM urine, 0.4 μM (serum)

OTHER SUBSTANCES
Extracted: acetylaminosalicylic acid

KEY WORDS
serum; pharmacokinetics; derivatization

REFERENCE
Ryde,E.M.; Ahnfelt,N.-O. The pharmacokinetics of olsalazine sodium in healthy volunteers after a single i.v. dose and after oral doses with and without food, *Eur.J.Clin.Pharmacol.*, **1988**, *34*, 481–488.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 500 μL Plasma + 500 μL 100 mg/mL (sic) 2,4-dihydroxybenzoic acid in MeOH, mix, centrifuge, inject an aliquot. Urine. 100 μL Urine + 500 μL 600 mg/mL (sic) 2,4-dihydroxybenzoic acid in MeOH, mix, centrifuge, inject an aliquot.

HPLC VARIABLES
Guard column: μBondapak C18 Guard Pak
Column: 250 × 4.1 10 μm Caulab CSC PRP-1
Mobile phase: MeCN:MeOH:50 mM pH 7.9 potassium phosphate buffer 10:10:80
Flow rate: 1

Detector: F ex 315 em 475

CHROMATOGRAM
Retention time: 5.1
Internal standard: 2,4-dihydroxybenzoic acid (3.3)
Limit of quantitation: 1000 ng/mL (urine), 25 ng/mL (plasma)

OTHER SUBSTANCES
Extracted: N-acetyl-5-aminosalicylic acid

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Corey,A.E.; Rose,G.M.; Conklin,J.D. Bioavailability of single and multiple doses of enteric-coated mesalamine and sulphasalazine, *J.Int.Med.Res.*, **1990**, *18*, 441–453.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 205.2

CHROMATOGRAM
Retention time: 4.737

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: bulk
Sample preparation: Dissolve in dilute HCl (pH 2), sonicate if necessary, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Hypersil-BDS C18
Mobile phase: MeOH:THF:buffer 11:4:85 (Buffer was 8.6 g $NaH_2PO_4.H_2O$, 8.2 g NaCl, 2.2 g sodium 1-heptanesulfonate, and 5.75 mL 85% phosphoric acid in 2 L water, pH 2.0.)
Column temperature: 35
Flow rate: 1.5

Injection volume: 20
Detector: UV 300 or 215

CHROMATOGRAM
Retention time: 3.6

OTHER SUBSTANCES
Simultaneous: impurities, salicylic acid

REFERENCE
Kersten,B.S.; Catalano,T.; Rozenman,Y. Ion-pairing high-performance liquid chromatographic method for the determination of 5-aminosalicylic acid and related impurities in bulk chemical, *J.Chromatogr.*, **1991**, *588*, 187–193.

SAMPLE
Matrix: formulations
Sample preparation: 75 μL Sample + 6 mL mobile phase, vortex for 2 min, add 300 μL 300 μg/mL acetaminophen, make up to 10 mL with mobile phase, mix for 2 min, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spheri-5 ODS (Applied Biosystems)
Mobile phase: MeOH:buffer 30:70 (Buffer was 900 mL 50 mM Na_2HPO_4 + 18.75 mL tetrabutylammonium phosphate, pH adjusted to 6.8 with 1 N phosphoric acid.)
Flow rate: 1.2
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Retention time: 6.0
Internal standard: acetaminophen (3.8)

KEY WORDS
stability-indicating; rectal suspension; enema

REFERENCE
Henderson,L.M.; Johnson,C.E.; Berardi,R.R. Stability of mesalamine in rectal suspension diluted with distilled water, *Am.J.Hosp.Pharm.*, **1994**, *51*, 2955–2957.

SAMPLE
Matrix: microsomal incubations
Sample preparation: 250 μL Microsomal incubation + 500 μL MeOH, centrifuge, inject an aliquot of the supernatant.

HPLC VARIABLES
Column: 120 × 4.6 5 μm RP-18
Mobile phase: MeCN:200 mM pH 7.5 phosphate buffer:1.25 mM cetyltrimethylammonium bromide 30:5:65
Flow rate: 1
Detector: F ex 315 em 500

KEY WORDS
rat; for 5-aminosalicylic acid-O-sulfate and 5-aminosalicylic acid

REFERENCE
Herzog,R.; Leuschner,J. Experimental studies on the pharmacokinetics and toxicity of 5-aminosalicylic acid-O-sulfate following local and systemic application, *Arzneimittelforschung*, **1995**, *45*, 300–303.

SAMPLE
Matrix: tissue

Sample preparation: Freeze mucosal intestinal biopsy (ca. 5 mg) in liquid nitrogen and crush the sample, allow to warm to room temperature, add 20 μL propionic anhydride, add 100 μL 57.6 μg/mL N-propionyl-4-aminosalicylic acid (purified by HPLC) in mobile phase, add 500 μL 50 mM pH 7.4 phosphate buffer, wash grinding/mixing rod with 500 μL 50 mM pH 7.4 phosphate buffer, sonicate (80 W) by immersing microprobe tip in mixture for 1 min, vortex, let stand at 37° for 1 h, add 500 μL 10% NaCl, add 6 mL MeCN, extract, cool at 4° for 1 h, centrifuge at 1500 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of air, reconstitute the residue in 500 μL mobile phase, filter (0.45 μm), inject a 100 μL aliquot.

HPLC VARIABLES
Guard column: 10 × 4.6 5 μm Spherisorb ODS-2
Column: 150 × 4.6 3 μm Spherisorb ODS-2
Mobile phase: MeCN:100 mM acetic acid:triethylamine 8:92:0.2
Flow rate: 1.5
Injection volume: 100
Detector: F ex 315 em 430

CHROMATOGRAM
Retention time: 5.7
Internal standard: N-propionyl-4-aminosalicylic acid (7.3)
Limit of detection: 1 ng

OTHER SUBSTANCES
Extracted: N-acetyl-5-aminosalicylic acid

KEY WORDS
mucosal intestinal biopsy; derivatization

REFERENCE
De Vos,M.; Verdievel,H.; Schoonjans,R.; Beke,R.; De Weerdt,G.A.; Barbier,F. High-performance liquid chromatographic assay for the determination of 5-aminosalicylic acid and acetyl-5-aminosalicylic acid concentrations in endoscopic intestinal biopsy in humans, *J.Chromatogr.*, **1991**, *564*, 296–302.

SAMPLE
Matrix: tissue
Sample preparation: Sonicate tissue in 2 mL 5 ng/mL 3,4-dihydroxybenzylamine in MeOH for two 30 s cycles (W = 60), centrifuge at 1800 g for 10 min. Filter (0.5 μm) the supernatant, evaporate the filtrate to dryness under a stream of nitrogen under reduced pressure, reconstitute with 100 μL mobile phase, vortex, inject a 5 μL aliquot.

HPLC VARIABLES
Guard column: 50 × 4.6 40 μm Pelliguard (Supelco)
Column: 250 × 4.6 10 μm Erbasil S C18 (Carlo Erba)
Mobile phase: MeOH:buffer 15:85, pH adjusted to 3 with 100 mM NaOH. (Buffer was 10 mM Na_2HPO_4 containing 0.1 mM EDTA, 100 mM citric acid, and 0.1 mM heptanesulfonic acid.)
Flow rate: 1
Injection volume: 5
Detector: E, ESA Model 5100A, Model 5021 conditioning cell +0.35 V (between column and analytical cell), Model 5011 analytical cell, first electrode +0.05 V, second electrode -0.50 V (monitored)

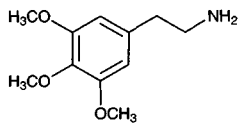
CHROMATOGRAM
Retention time: 3.2
Internal standard: 3,4-dihydroxybenzylamine (4.4)
Limit of detection: 1 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

REFERENCE

Palumbo,G.; Carlucci,G.; Mazzeo,P.; Frieri,G.; Pimpo,M.T.; Fanini,D. Simultaneous determination of 5-aminosalicylic acid, acetyl-5-aminosalicylic acid and 2,5-dihydroxybenzoic acid in endoscopic intestinal biopsy samples in humans by high-performance liquid chromatography with electrochemical detection, *J.Pharm.Biomed.Anal.*, **1996**, *14*, 175–180.

Mescaline



Molecular formula: $C_{11}H_{17}NO_3$
Molecular weight: 211.26
CAS Registry No.: 54-04-6
Merck Index: 5965

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

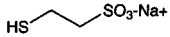
OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primi-

done, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE
Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Mesna



Molecular formula: $C_2H_5NaO_3S_2$
Molecular weight: 164.18
CAS Registry No.: 19767-45-4, 3375-50-6 (free acid)
Merck Index: 5969

SAMPLE
Matrix: blood
Sample preparation: 50 μL Plasma (within 3 min of collection) + penicillamine + 10 μL 25 mM monobromobimane in MeCN, let stand for 5 min at room temperature, add 20 μL 20% perchloric acid. (For total mesna add 100 μL 20 mM dithiothreitol in 200 mM pH 8.5 Tris/HCl buffer to 50 μL plasma, let stand for 40 min at room temperature, add 50 μL 15% sulfosalicylic acid, centrifuge. Remove 200 μL of the supernatant and wash it three times with ethyl acetate. Remove 60 μL of the aqueous phase and add it to 300 μL 200 mM pH 8.5 Tris/HCl buffer and 10 μL 15 mM monobromobimane in MeCN, let stand at room temperature for 5 min, add 20 μL 20% perchloric acid.)

HPLC VARIABLES
Column: 150 × 4.6 7 μm Nucleosil RP-18
Mobile phase: Gradient. A was MeCN. B was 1% aqueous acetic acid containing 1 g/L octanesulfonic acid. A:B from 5:95 to 8:92 over 2 min, to 10:90 over 13 min (Waters convex), to 30:70 over 20 min (Waters convex), maintain at 30:70 for 6 min, re-equilibrate at initial conditions for 9 min.
Flow rate: 1.4
Detector: F (wavelengths not given)

CHROMATOGRAM
Internal standard: penicillamine
Limit of detection: 10 μM

KEY WORDS
plasma; derivatization; pharmacokinetics

REFERENCE
Stofer-Vogel,B.; Cerny,T.; Borner,M.; Lauterburg,B.H. Oral bioavailability of mesna tablets, *Cancer Chemother.Pharmacol.*, **1993**, *32*, 78–81.

SAMPLE
Matrix: blood, urine

Sample preparation: Plasma. 400 μL Plasma + 400 μL 333 mM sulfuric acid + 400 μL 5% sodium hexametaphosphate, vortex for 5 s, centrifuge at 15000 g for 2 min, inject a 20 μL aliquot of the supernatant. (Process plasma as rapidly as possible after collection.) (For total mesna mix 100 μL plasma, 100 μL EDTA solution, and 100 μL 4% sodium borohydride, heat to 50° for 30 min, cool, add 200 μL 9.7% acetic acid, store in the dark for 3-4 days, inject an aliquot. (EDTA solution was 1% disodium EDTA in a mixture of 50 mL 500 mM Na_2HPO_4 and 22 mL 1 M NaOH.)) Urine. Dilute 1:10 to 1:400 with mobile phase, inject an aliquot. (For total mesna dilute 1:10 to 1:1000 then proceed as above.)

HPLC VARIABLES

Column: 100 × 4.6 3 μm Hypersil ODS
Mobile phase: MeCN:buffer 3:97 (Buffer was 100 mM pH 7.0 phosphate buffer containing 5 mM n-tetrabutylammonium hydroxide.)
Flow rate: 1
Injection volume: 20
Detector: E, Coulochem Model 5100A, Model 5011 analytical cell, detector 1 not used, detector 2 -200 mV

CHROMATOGRAM

Retention time: 4.5
Limit of quantitation: 6.1 μM

KEY WORDS

plasma

REFERENCE

James,C.A.; Rogers,H.J. Estimation of mesna and dimesna in plasma and urine by high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.*, **1986**, *382*, 394–398.

SAMPLE

Matrix: blood, urine
Sample preparation: Plasma. 100 μL Plasma + 25 μL 330 mM sulfuric acid + 25 μL 5% sodium hexametaphosphate + 10 μL 40 μg/mL p-aminobenzoic acid, make up to 200 μL, vortex for 2 s, centrifuge at 3400 g for 6 min, inject a 50-100 μL aliquot of the supernatant. Urine. Dilute 1:50 with water. 100 μL Diluted urine + 100 μL 150 μg/mL p-aminobenzoic acid in 1.25% sodium hexametaphosphate, vortex for 2 s, inject a 50 μL aliquot. (Reduce dimesna to mesna as follows. 100 μL Plasma or diluted urine + 100 μL 1% EDTA in buffer + 100 μL 1.06 M sodium borohydride in water, mix thoroughly, heat at 50° for 30 min, cool to room temperature, add 200 μL 1.74 M acetic acid, vortex for 20 s, centrifuge for 10 min, inject a 50-100 μL aliquot of the supernatant. Buffer was 50 mL 500 mM Na_2HPO_4 + 22 mL NaOH.)

HPLC VARIABLES

Guard column: Guard PAK C18 (Waters)
Column: 100 × 8 10 μm Resolve C18 (Waters)
Mobile phase: 100 mM Sodium citrate containing 1 mM tetrabutylammonium phosphate and 0.71 mM triethylamine, pH adjusted to 5 with 85% phosphoric acid
Flow rate: 2
Injection volume: 50-100
Detector: E, ESA Coulochem II model 5100, model 5011 analytical cell +450 mV, model 5021 conditioning cell +500 mV

CHROMATOGRAM

Retention time: 6.73
Internal standard: p-aminobenzoic acid (8.87)
Limit of quantitation: 1 μg/mL

OTHER SUBSTANCES

Simultaneous: acetaminophen, 5-fluorouracil, procarbazine, prochlorperazine
Noninterfering: aspirin, bleomycin, carboplatin, carmustine, chlorambucil, cyclophosphamide, cyclosporine A, cytarabine, doxorubicin, etoposide, hydrocortisone, ifosfamide, lomustine, methotrexate, mitomycin, mitoxantrone, teniposide, thiotepa, vincristine

KEY WORDS
plasma; pharmacokinetics

REFERENCE
el-Yazigi,A.; Yusuf,A.; Al-Rawithi,S. Liquid chromatographic analysis of mesna and dimesna in plasma and urine of patients treated with mesna, *Ther.Drug Monit.*, **1995**, *17*, 153–158.

SAMPLE
Matrix: formulations
Sample preparation: Dilute formulation with 15 μg/mL disodium EDTA solution to a mesna concentration of 3.2 μg/mL. Remove a 1 mL aliquot and add it to 300 μL reagent solution, let stand at room temperature for 20 min, add 500 μL 300 mM phosphoric acid solution, add 3 mL 4 μg/mL IS solution, make up to 10 mL with water, inject a 50 μL aliquot. (Prepare the reagent solution by dissolving 3.5 mg methyl 4-(6-methoxynaphthalen-2-yl)-4-oxo-2-butenoate in 10 mL THF, make up to 25 mL with pH 7.5 borate buffer. Prepare methyl 4-(6-methoxynaphthalen-2-yl)-4-oxo-2-butenoate as follows. Dissolve 5 g 6'-methoxy-2'-acetonaphthone in warm glacial acetic acid and add 2.5 g glyoxylic acid, reflux for 24 h, evaporate to dryness under reduced pressure. Take up the residue in chloroform and extract it three times with 5% sodium carbonate solution. Combine the aqueous layers and acidify them with concentrated HCl, collect the product by filtration, recrystallize from MeOH/water or acetic acid to give 4-(6-methoxynaphthalen-2-yl)-4-oxo-2-butenoic acid (mp 167-9°) (Farmaco, Ed. Sci. 1982, 37, 171). Reflux 0.5 g 4-(6-methoxynaphthalen-2-yl)-4-oxo-2-butenoic acid, 2.5 mL MeOH, and 2-3 drops sulfuric acid in 25 mL anhydrous benzene (Caution! Benzene is a carcinogen!) for 1 h, add 20 mL water, wash the organic layer with 10 mL 5% sodium bicarbonate solution, wash the organic layer with 20 mL water. Dry the organic layer over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, purify by flash chromatography on silica gel using ethyl acetate:light petroleum (bp 40-70°) 40:60 to give methyl 4-(6-methoxynaphthalen-2-yl)-4-oxo-2-butenoate as a pale yellow compound (mp 116-120°).)

HPLC VARIABLES
Column: 150 × 4 5 μm Spherisorb RP-8
Mobile phase: MeOH:50 mM pH 3.0 triethylammonium phosphate 53:47
Flow rate: 1
Injection volume: 50
Detector: F ex 310 em 450

CHROMATOGRAM
Retention time: 6
Internal standard: 4-(6-methoxynaphthalen-2-yl)-4-oxobutanoic acid (8)

OTHER SUBSTANCES
Simultaneous: acetylcysteine, cysteamine, cysteine, glutathione, homocysteine
Noninterfering: bacitracin, biotin, calcium pantothenate, cystine, glycine, magnesium oxide, neomycin, starch, threonine, vitamin E, pyridoxine, riboflavin phosphate

KEY WORDS
solutions; derivatization

REFERENCE
Gatti,R.; Cavrini,V.; Roveri,P.; Pinzauti,S. High-performance liquid chromatographic determination of aliphatic thiols with aroylacrylic acids as fluorogenic precolumn derivatization reagents, *J.Chromatogr.*, **1990**, *507*, 451–458.

SAMPLE
Matrix: formulations
Sample preparation: Dilute with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm cyano
Mobile phase: MeCN:100 mM NaH_2PO_4 20:80 adjusted to pH 4.2 with phosphoric acid
Flow rate: 2.5

Injection volume: 20
Detector: UV 200

CHROMATOGRAM
Retention time: 1.16

OTHER SUBSTANCES
Simultaneous: diphenhydramine, granisetron (UV 300)

KEY WORDS
stability-indicating; injections; saline

REFERENCE
Mayron,D.; Gennaro,A.R. Stability and compatibility of granisetron hydrochloride in i.v. solutions and oral liquids and during simulated Y-site injection with selected drugs, *Am.J.Health-Syst.Pharm.*, **1996**, *53*, 294–304.

SAMPLE
Matrix: urine
Sample preparation: Dilute urine 1:3 to 1:39, inject a 50 μL aliquot.

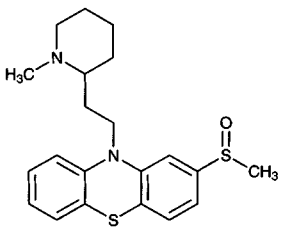
HPLC VARIABLES
Column: 250 × 4 5 μm Hypersil ODS
Mobile phase: MeOH:250 mM pH 7.4 phosphate buffer 5:95 containing 5 mM tetrabutylammonium phosphate
Flow rate: 1
Injection volume: 50
Detector: UV 412 following post-column reaction. The column effluent mixed with the reagent pumped at 0.5 mL/min and the mixture flowed through a 200 × 4 column packed with 100-120 mesh glass beads (dichlorodimethylsilane treated) to the detector. (Prepare reagent by diluting 0.2% 5,5'-dithiobis(2-nitrobenzoic acid) in 250 mM pH 7.4 phosphate buffer containing 10% tripotassium citrate 1:10 with water.)

CHROMATOGRAM
Retention time: 7.5
Limit of detection: 75 ng

KEY WORDS
post-column reaction; comparison with electrochemical detection without derivatization

REFERENCE
Sidau,B.; Shaw,I.C. Determination of sodium 2-mercaptoethanesulphonate by high-performance liquid chromatography using post-column reaction colorimetry or electrochemical detection, *J.Chromatogr.*, **1984**, *311*, 234–238.

Mesoridazine



Molecular formula: $C_{21}H_{26}N_2OS_2$
Molecular weight: 386.58
CAS Registry No.: 5588-33-0, 32672-69-8 (besylate)
Merck Index: 5970
Lednicer No.: 1 389

SAMPLE
Matrix: blood
Sample preparation: 2 mL Plasma + 100 μL 1 M HCl, vortex for 30 s, add 4 mL isopropanol, mix for 5 min, centrifuge at 5000 rpm at 0° for 20 min. Remove the supernatant and adjust